

MORPHOMETRIC VARIATION AMONG POPULATIONS OF *AMBRYsus MORMON* MONTANDON (HETEROPTERA: NAUCORIDAE)

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Abstract.—Morphometric variation with respect to 15 mensural characters was assessed for adult specimens from 13 populations of *Ambrysus mormon* Montandon in the United States. This provides a context from which to assess the degree of divergence of an isolated population (Ash Warm Springs, Nevada), which possesses discrete-state characteristics that differ markedly from those of other populations of the species. A multivariate analysis of variance revealed that interpopulational differences were dependent on sex, a finding corroborated by the univariate perspective provided by Bonferroni's sequential adjustment that additionally identified lengths of body, protibia, and protarsus as contributing to the interaction between population and sex. Moreover, all pairwise comparisons of the 13 populations were significant for males as well as for females (*F*-tests from discriminant function analysis). Indeed, the pattern of morphometric variation among populations was similar in males and females but was not a consequence of differentiation by geographic distance (Mantel analyses). Interindividual variation primarily was due to differences in size, as the first axis from principal components analysis accounted for 92.2% of the total variation. Three additional axes represented shape, and each accounted for at least 10% for the remaining variation among individuals. The population from Ash Warm Springs differed markedly (larger protarsus relative to meso- and metatarsi, and smaller pro-, meso-, and metatarsi relative to body length and synthlipsis) from the other populations, suggesting the need for systematic revision of its subspecific affiliation. In contrast, populations of *A. m. heidemanni* Montandon and *A. m. minor* La Rivers were similar to the other populations of the nominate subspecies, suggesting their subspecific recognition is questionable. The distinctiveness of the population from Ash Warm Springs as an isolated remnant of the historically connected pluvial White River intimates that it may be a taxon in the process of specific differentiation.

Key Words: Naucoridae, *Ambrysus mormon*, morphometrics, phenetics, shape

Ambrysus mormon Montandon is the most widespread species of the genus in the United States, ranging from Oregon and Idaho east to South Dakota, and south through Arizona and New Mexico into

Mexico. Throughout its range, *A. mormon* occurs in diverse habitats ranging from cold montane streams to thermal spring effluents. Typically, it is found in slow water near margins of gravel-bottomed streams

(Usinger 1946). Four subspecies currently are recognized (La Rivers 1971): *A. m. australis* La Rivers in Mexico, southern Texas, and New Mexico; *A. m. heidemanni* Montandon in thermal runoffs in Yellowstone National Park; *A. m. minor* La Rivers in a thermal spring in Idaho; and the nominate subspecies throughout the remainder of the range in the United States from Idaho and South Dakota south to Arizona and New Mexico.

Because of their aquatic habitat requirements, occurrence in otherwise arid landscapes, and limited dispersal abilities, naucorids in the southwestern United States are characterized by a high level of endemism. For example, *A. relictus* Polhemus and Polhemus and *A. amargosus* La Rivers, which is listed as a federally endangered species, occur only in several fragile streams in a western Nevada desert oasis; *A. funebris* La Rivers occurs only in Death Valley; and *Limnocois moapensis* (La Rivers) occurs only in the vicinity of Moapa, Nevada.

An isolated population of *A. mormon* at Ash Warm Springs, Nevada, possesses discrete morphological characteristics that differ from those of other populations of the species. As a parallel study to an analysis of discrete characters (J. T. Polhemus, in litt.), we present an analysis of shape-related variation in *A. mormon* to determine the degree of divergence of the Ash Warm Springs population from other populations of the nominate subspecies, as well as from *A. m. minor* and *A. m. heidemanni*.

MATERIALS AND METHODS

A suite of 15 external mensural characters (body length and width; head length and width; synthlipsis; pronotal length; lengths of pro-, meso-, and metathoracic femur, tibia, and tarsus), previously determined to be effective in discriminating among naucorid taxa (Sites and Willig 1994a, b), was measured for adult specimens of 13 populations of *A. mormon* (Table 1, Fig. 1). Body length was measured from the tip of the labrum to tip of the ab-

domen; body width, head length, head width, and all leg segments were longest distances; pronotal length was measured along the midline. Meso- and metanotal lengths were not included because it is difficult to obtain accurate measurements without dissection of specimens. Each of these nota subducts below the preceding notum, and the visible length is variable and dependent on the degree of thoracic flexion. Generally, 10 specimens of each sex from each population were measured; however, for four populations, fewer than 10 specimens were available (Eel River, Hot Creek Falls, Utah, and Yellowstone). All data were transformed to natural logarithms to evaluate more effectively the contribution of shape (see Sites and Willig 1994a, b) to interpopulational differences. Statistical analyses were executed using SPSS (1990). Voucher specimens are deposited in the Enns Entomology Museum, University of Missouri-Columbia, and the John T. Polhemus Collection.

Data from labels of 18 of the 19 specimens of *A. m. minor* provided insufficient detail concerning site of collection (Table 1, HCF) to ascribe individuals to the type locality for the subspecies with certainty. More specifically, a single specimen from the type locality of Hot Creek Falls is included in the analysis, along with a series of 18 specimens labeled "nr. Bruneau." Therefore, the morphometric affinities of these specimens with that of the known topotypic specimen were determined using discriminant function analysis with groups defined by combinations of sex and population.

Two-way multivariate analysis of variance (MANOVA) evaluated differences among populations and between sexes based on mensural characters. Univariate, two-way analyses of variance (ANOVAs) were performed to assess the contribution of each particular character to multivariate group differences. To minimize the likelihood of overestimating the significance of individual characters that compose a large

Table 1. Acronyms and collection data for populations of *Ambrysus mormon*. Numbers of measured male and female specimens, respectively, appear parenthetically below each acronym.

AWS (10,10)	NEVADA: Lincoln Co. Ash Warm Springs 3750 ft. elev.; 36°C 21 July 1992; CL 2711 J. T. and D. A. Polhemus	MOA (10,10)	NEVADA: Clark Co. Warm Springs; 32°C 27 August 1989 coll: J. A. Back
COL (10,10)	COLORADO: Pueblo Co. Burnt Mill Creek CL2683; 19-V-1992 J. T. Polhemus	NMX (10,10)	NEW MEXICO: Lincoln Co. Rio Hondo 2 mi E Hondo 23 Sept. 1988; 12°C coll: R. W. Sites veg. in flow at margin
EEL (8,10)	CALIFORNIA: Mendocino Co. Eel River at Bell glen nr. Leggett 62°F; 2-VII-1959 R. K. Allen	NYE (10,10)	NEVADA: Nye Co. Hot Creek Spring 7 mi. W. Hwy 93 30°C; CL2898 coll: J. T. and D. A. Polhemus
HCF (0,1)	IDAHO: Owyhee Co. (1) Hot Cr. Falls 9-IX-1965 E. J. Allen	SDA (10,10)	SOUTH DAKOTA: Fall River Co. Hot Springs; 2 mi NW Hot Water 22 June 1940; H. C. Severin
HCF (10,8)	IDAHO: Owhyee Co. (18) nr. Bruneau 10-X-1975 A. D. Allen	SHA (10,10)	CALIFORNIA: Shasta Co. Rt. 44; Cow Ck nr Palo Cedro 28 Aug 1991; R. S. Zack and M. A. Valenti, collrs.
IDA (10,10)	IDAHO: Owyhee Co. Bruneau Riv. ca. 7 mi SSE Bruneau T7S R6E sect. 26 22 Sept. 1988 R. S. Zack, coll.	UTA (10,8)	UTAH: Washington Co. North Creek nr. Virgin 16-X-1982 D. A. Polhemus
INY (10,10)	CALIFORNIA: Inyo Co. Shoshone Hot Spring CL 2904 coll: J. T. and D. A. Polhemus	YST (9,10)	WYOMING: Yellowstone Nat. Pk. CL 556; 15-VIII-1972 J. T. Polhemus

suite of attributes (Holm 1979, Rice 1989), the Bonferroni sequential adjustment was applied to each morphometric character before ascribing statistical significance to univariate analyses. Discriminant function analysis (DFA) simultaneously maximized intergroup differences and minimized intra-group variation among individuals by adjusting the linear combination of variables in each of a number of orthogonal axes. Pairwise *F*-tests associated with DFA determined which populations differed significantly from each of the other populations.

The subsequent classification phase of DFA then assigned each specimen to a population based on the linear combination of variables from each discriminant function axis. Percent of correct assignments was used as a separate measure of morphometric distinction among populations. Because DFA reconstitutes variables to maximize differences among groups, natural relationships can become distorted in multidimensional space. In contrast, principal components analysis (PCA) was used as a data reduction technique to retain natural distance relations



Fig. 1. Range (shaded) of *Ambrysus mormon* in the United States and localities (black circles) represented in analyses.

among individuals in multidimensional space and to view differences among populations with regard to shape and size for males and females separately. If the magnitude of differences in shape among populations is a consequence of the degree of isolation derived from geographic distance, then a correlation should exist between a matrix of pairwise linear distances between sites and a matrix of pairwise morphometric distances based on population centroids (PC2 through 4). We tested this hypothesis of differentiation by distance separately for

males and females using Mantel correlation analysis (Fortin and Gurevitch 1993, Manly 1994, Sokal and Rohlf 1995). Via a separate Mantel analysis, we evaluated the degree to which interpopulational differences in shape between males and females were similar, regardless of geographic correlates.

RESULTS

The 18 specimens labeled “nr. Bruneau” of uncertain subspecific association likely represent *A. m. minor* because they formed a well-defined cluster in morphometric

Table 2. Significance levels (*P*)^a of the character suite (MANOVA) and each character separately (ANOVA) in distinguishing between sexes and among 13 populations of *Ambrysus mormon*.

	Population	Sex	Pop × Sex
MANOVA	<0.001	<0.001	<0.001
ANOVA			
Body length	<0.001	<0.001	0.001
Body width	<0.001	<0.001	0.180 ns
Head length	<0.001	<0.001	0.908 ns
Head width	0.001	<0.001	0.922 ns
Synthlipsis	<0.001	<0.001	0.121 ns
Pronotum	<0.001	<0.001	0.418 ns
Profemur	<0.001	<0.001	0.099 ns
Protibia	<0.001	<0.001	<0.001
Protarsus	<0.001	<0.001	0.003
Mesofemur	<0.001	<0.001	0.427 ns
Mesotibia	0.001	<0.001	0.353 ns
Mesotarsus	<0.001	<0.001	0.099 ns
Metafemur	<0.001	<0.001	0.018 ns
Metatibia	<0.001	<0.001	0.009 ns
Metatarsus	<0.001	<0.001	0.437 ns

^a Bonferroni sequential adjustments within a column for the 15 univariate characters corroborated significant ($\alpha \leq 0.05$) group differences in all situations except those marked ns.

space that was distinct from that of specimens representing the population of *A. m. mormon* in the Bruneau River (IDA). In addition, each specimen was assigned to the correct population in the classification phase of DFA, further substantiating the morphometric distinction of these specimens. All 19 specimens match discrete characteristics listed for the subspecies by La Rivers (1963) and are small; this is typical of naucorids that develop in high environmental temperatures (Sites et al. 1996). Differences among populations (Table 2) depended on sex (MANOVA, Population by Sex interaction, $P \leq 0.001$). Three characters (lengths of body, protibia, and protarsus) contributed significantly ($P \leq 0.05$) to the interaction (Table 2). All other characters contributed significantly to differences ($P \leq 0.001$) among populations or between sexes in a consistent fashion.

Pairwise *F*-tests revealed very highly significant morphometric differences ($P \leq$

0.001) between each possible pair of populations (78 pairwise contrasts per sex). The classification phase of DFA assigned all but two (98.43%) male specimens and all but one (99.21%) female specimen to the correct population. Clearly, intrapopulational morphometric variation was considerably less than interpopulational differences.

Principal components analysis effectively disassociated aspects of size (PC 1) and shape (other axes) from morphological variation among individuals. The loading coefficient of each character was positive on PC 1 (Table 3) and accounted for 92.2% of the interindividual variation. Three additional axes each accounted for at least 10.0% of the shape-related variation. Of these shape axes, PC2, PC3, and PC4 accounted for 33.5%, 20.5%, and 10.4% of the shape-related variation among individuals, respectively. Males and females show correlated patterns of interpopulational variation in shape (Mantel analysis; $P = 0.015$). This is reinforced by results from PCA in which the interpopulational dispersion with respect to shape is similar in males and females (Fig. 2). The main difference between the sexes is captured by PC2 (lengths of meso- and metatarsi relative to protarsus): females from a particular population have larger scores on PC2 than do corresponding males. Nonetheless, the degree of differentiation in shape was unrelated to geographic distance between populations for males (Mantel analysis; $P = 0.604$) and females (Mantel analysis; $P = 0.278$). Factors other than distance, per se, must be affecting interpopulational variation in shape in this species.

Regardless of sex, the attribute of shape that distinguishes the Ash Warm Springs population from the other populations of *A. mormon* is its relatively elongate protarsi compared to meso- or metatarsi (PC2). To a lesser degree, this same attribute of shape distinguished populations of MOA and NYE from the remainder of populations, especially when considering females. Addi-

Table 3. Principal component loadings (correlations) for axes representing size (PC1) and appreciable attributes of shape (PC2 through 4).

Character	PC1	PC2	PC3	PC4
Body length	0.964	-0.031	-0.143	-0.168
Body width	0.986	-0.006	-0.057	-0.113
Head length	0.978	-0.019	-0.074	-0.024
Head width	0.977	-0.006	-0.058	-0.006
Synthlipsis	0.925	0.164	-0.246	0.109
Pronotum length	0.964	-0.098	-0.108	-0.006
Profemur length	0.973	-0.054	-0.011	0.175
Protibia length	0.970	-0.099	0.014	0.174
Protarsus length	0.883	-0.338	0.258	-0.002
Mesofemur length	0.992	-0.014	-0.007	0.002
Mesotibia length	0.986	-0.068	0.046	-0.006
Mesotarsus length	0.880	0.398	0.203	0.020
Metafemur length	0.992	-0.016	0.009	-0.044
Metatibia length	0.985	-0.037	0.065	-0.063
Metatarsus length	0.936	0.250	0.144	-0.040
Eigenvalue	13.826	0.393	0.240	0.122
% variance (total)	0.922	0.026	0.016	0.008
% variance (shape)	—	0.335	0.205	0.104

tional shape variation that distinguishes all populations (PC3) is related to length of tarsi relative to body size and synthlipsis. In particular, the relative lengths of tarsi in the three populations from White River (AWS, MOA, and NYE) are smaller than those in the other populations.

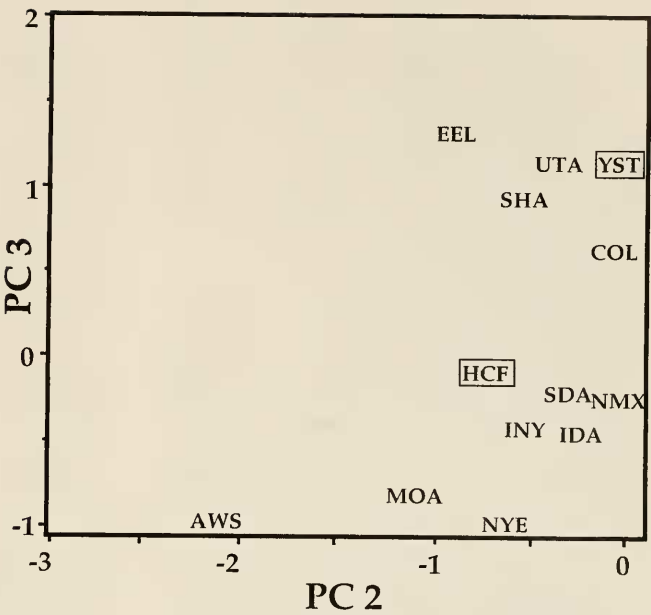
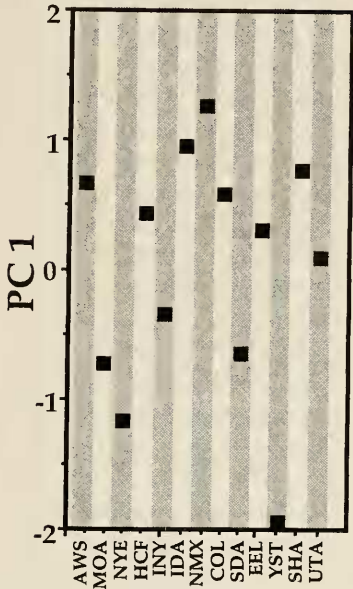
DISCUSSION

Recently, Sites and Willig (1994a, b) demonstrated that the above character suite was effective at discriminating among genera and species of naucorids. This character suite is not only diacritical in distinguishing among taxa but discriminates among conspecific populations of *A. mormon*. Nonetheless, the particular components of shape that distinguished 22 species of *Ambrysus* (Sites and Willig 1994a) are different than those that distinguished populations of *A. mormon*. The positions of populations of *A. mormon* in multidimensional morphometric space do not reflect currently established subspecific designations within the species. More specifically, *A. m. heidemanni* (YST) and *A. m. minor* (HCF) occur in close morphometric proximity to members of the nominate subspecies (all other populations),

and occur within the range of shape variability encompassed by the nominate subspecies (Fig. 2). Moreover, populations of the nominate subspecies from Ash Warm Springs, Moapa, and New Mexico (AWS, MOA, NMX) are considerably more divergent based on shape-related variation (Fig. 2) than either of the two other currently recognized subspecies included in this analysis (*A. m. heidemanni*, YST; *A. m. minor*, HCF). Further, discrete characters defining *A. m. heidemanni*, which occurs in the warm waters of the Yellowstone geyser basins, may be expressed in other warm water populations of nominate *A. mormon*, which led La Rivers (1951) to question the subspecific affiliation of this population. Because shape-related variation among populations does not reflect the currently accepted intraspecific taxonomy of *A. mormon*, and discrete characters offer only equivocal support, re-evaluation of subspecific status is warranted for those populations.

Populations from Utah, Yellowstone, Colorado, and South Dakota; as well as those from Idaho and New Mexico (Sites et al. 1996), reflect a wide range of envi-

Males



Females

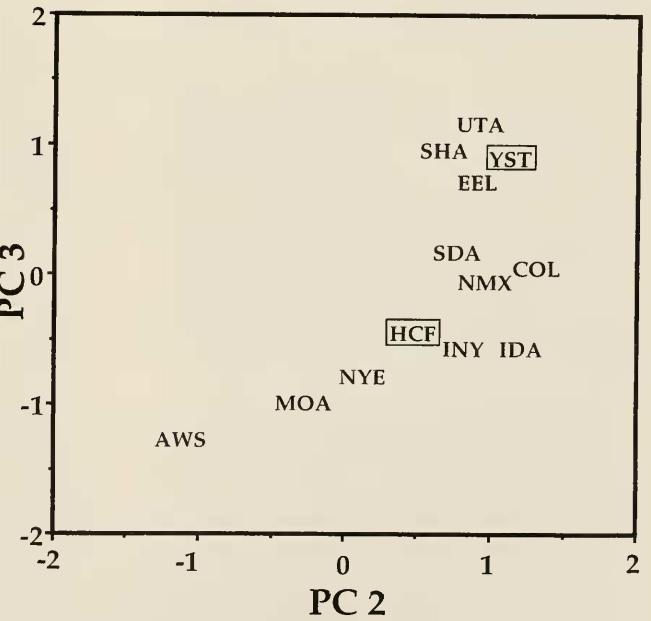
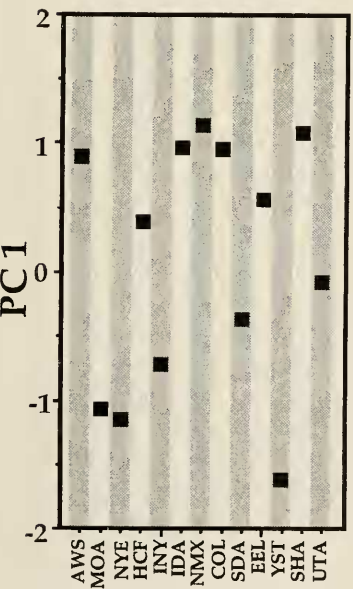


Fig. 2. Plots of 13 population centroids, derived from a principal components analysis of 15 mensural characters, illustrate intraspecific size (PC1) and shape (PC 2 and 3) variation for male and for female *A. mormon*. Eleven populations represent nominate *A. mormon*, whereas HCF and YST populations (in boxes) represent *A. m. minor* and *A. m. heidemannii*, respectively.

ronmental temperatures during development, yet the positions of group centroids (Fig. 2) indicate that these populations share similar attributes of shape. Although thermal environment does influence allometric relationships during ontogeny (Sites et al. 1996), the greatest degree of shape-related variation in *A. mormon* has been achieved independently of thermal environment during ontogeny.

The population of *A. m. minor* (HCF) exhibits morphometric attributes distinct from those of the nearby population of nominate *A. mormon* in the Bruneau River (IDA), based on a pairwise *F*-test from DFA. Despite the close proximity of Hot Creek Falls to Bruneau River (≈ 1 km), distinct morphologies characterize the populations of the two sites. A series of unnamed hot springs occurs along the Bruneau River, and *A. mormon* may occur in them. Because of local irrigation demands, the water table has declined recently, resulting in a concomitant reduction in the size of the springs. During the last known visit to these springs (1991), the pool at the base of Hot Creek Falls, known as Indian Bathtub, was reduced tremendously in size (≈ 1.0 m diam $\times 0.3$ m depth) and persisted only because a piece of plastic had been placed on the downstream side of the pool, probably to allow bathing; the actual falls no longer exists (R. S. Zack, personal communication). Continued reduction in the water table may threaten *A. m. minor* to the point that it may soon become extinct.

The White River system in eastern Nevada extends from the White Pine Mountains near Ely south to the Moapa River near Lake Mead. The lower end of the White River near Moapa is known as the Muddy River. The White River system is of hydrographic and faunistic interest because of its recently restricted watercourse and isolated fish populations. In the Pliocene and at least the early Pleistocene epochs, the pluvial White River was continuous throughout its length to an isolated lake, now represented by the Moapa River (Gil-

bert 1893, Carpenter 1915), which persists as a tributary of the Colorado River. Since that time, extensive desertification of the Great Basin has occurred (King 1958), significantly reducing the extent of the White River. As a result, ≈ 200 miles of dry river bed now cross the parched desert, isolating the Recent White River (north) from the Moapa River (south). Despite drastic reductions in surface water, sporadically distributed springs and effluent fragments of the pluvial White River persist.

The fish fauna of the White River is distinct and includes an endemic cyprinid genus, an endemic species of *Crenichthys*, and several indigenous species and subspecies (Hubbs and Miller 1948). Subspecific affinities of isolated fish populations in these springs and effluents strongly suggest that these habitats were continuous in the past. *Ambrysus mormon* also occurs in many of these aquatic fragments, a result of its almost certain occurrence in the pluvial White River and subsequent isolation following landscape wide desertification. Three populations included in our morphometric assessment represent White River isolates [AWS, MOA, and NYE (see Table 1)]. Specimens of the Ash Warm Springs population exhibit structural features that are divergent from "typical" *A. mormon*, including reduction in posterolateral connexival spines, narrow embolium, and differences in genitalic features.

Evaluation of discrete-state characters of the isolated Ash Warm Springs population, combined with the attributes of shape of other members of the species, suggests that this population may warrant taxonomic recognition, at least at the subspecific level. Although the length of time that it has been separated from the parent population from White River is uncertain, clear differences in phenotype, including shape, have accumulated in isolation. Even if speciation is not yet complete, the population likely will continue to diverge from the other populations of *A. mormon* because of the effects of drift and natural selection in environ-

mental isolation. If divergence has not progressed to the point at which reproductive incompatibility exists between the Ash Warm Springs population and the remainder of *A. mormon*, then we are witnessing speciation in action in this remnant population of *A. mormon*. Although the Nye Co. and Moapa populations also represent isolates along the White River system, these populations have not diverged from typical morphometric form of *A. mormon* as radically as has the population at Ash Warm Springs (Fig. 2). Further studies involving discrete state characters are needed to resolve the intraspecific taxonomic status of these populations of *A. mormon*.

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